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NUTRITION REVIEWS

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NUTRITION REVIEWS

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ABERRANT LIPOGENESIS

The formation of microscopically visible fat by cells other than adipose tissue is called aberrant lipogenesis. This article outlines our experiments and interpretations of this process, summarizing papers published over the past several years. Although studied first and most extensively in the cornea, aberrant lipogenesis is a widespread phenomenon with, possibly, particular relevance to fatty degeneration and atheromatosis. It is a dynamic process of fat synthesis, culminating in glyceride esters, clearly distinguishable from phagocytosis of preformed fat (lipophanerosis).

Aberrant lipogenesis may be demonstrated *in vivo* by the injection of oleic, palmitic, or stearic acid into suitable tissues, but it is best induced by incubation of surviving tissue at 37°C in media containing salts of these fatty acids together with appropriately buffered serum. The fatty acid concentration (2 to 5 mg. per ml.) is critical; several-fold dilution will yield insignificant fat while excess will kill the tissue. The hydrogen ion concentration must be about neutrality (pH 6.8 to 8.0). The cornea is an especially favorable tissue because it has a relatively simple structure and yet contains components of epithelium, connective tissue and endothelium.

Incubation of corneal pieces in suitable media containing sodium oleate results in the formation of sudanophilic globules that appear in the cytoplasm first at six hours and increase progressively for several days. Through coalescence the globules eventually attain grotesque proprotions several times the size of the host cells. All the cells of the cornea (epithelium, stroma and endothelium) participate in this lipogenesis, yielding consistently reproducible results with successive experiments. If palmitic or stearic acid is substituted for oleic acid, the cells synthesize a lipid having the same distribution and the same time sequence but having a birefringent crystalline structure rather than sudanophilic globules.

With labeled fatty acids, it has been shown that the lipid formed is a neutral glyceride. Whether this product is mono-, di-, or triglycerides, or what proportion of each, has not yet been established.

Serum is essential to the process of aberrant lipogenesis. The precise role played by serum is not understood but both dialyzable and one or more non-dialyzable components are necessary. Calcium and magnesium may replace the former, but no one component (Cohn's fractions, lipoprotein, albumin, etc.) has been satisfactorily substituted for the latter. The serum factor is thermostable under conditions short of those which will coagulate the protein.

No significant accessory factors have been discovered that enhance the lipogenesis. Nucleotide co-factors and adenosine triphosphate have no effect when added to the medium. Addition of glucose and insulin or glycerol is similarly without effect. Cholesterol does not induce lipogenesis and does not increase that which occurs when suboptimal amounts of fatty acids are added (contrary to what has been found by others for tissue culture preparations). Occasionally hypercholesteremic serum from patients or rabbits will induce a small amount of fat formation, but this appears to be due to the increased availability of fatty acids rather than to the cholesterol.

Aberrant lipogenesis is an enzymatic and respiratory-dependent process. It is inactivated by prior heating of the tissue to 70° and is inhibited by para-chloromercuribenzoate, arsenite, or iodoacetic acid. It is sig-

nificantly diminished by sodium fluoride and potassium cyanide. Moreover, it appears to be a unidirectional process. There is no breakdown of the fat formed *in situ* and the lipid, when extracted and reinjected into the cornea *in vivo*, acts like a neutral fat that will be disposed of slowly by phagocytosis but will not induce lipogenesis. Yet to be studied is lipogenesis with separate subcellular particles. Identical results have been obtained with tissue from all animals tested (rabbit, cat, beef, mouse and man).

The tissue most profitably studied has been the cornea, but many tissues throughout the body are capable of aberrant lipogenesis. In general, the two types of tissue response are: (1) that typified by the cornea in which addition of fatty acids is mandatory for lipogenesis, and (2) that typified by the liver in which sufficient endogeneous substrates (presumably fatty acids) are present in the tissue to provide a built-in substrate for lipogenesis. The former type includes most connective tissue, arteries, and some epithelial membranes while the latter type includes parenchymatous organs such as the liver and kidney. Some tissues such as spleen, lymph nodes, white blood cells and adipose tissue (from starved animals) are not capable of aberrant lipogenesis under the experimental conditions.

A further difference between the tissues requiring the addition of fatty acids and those not requiring it is that the parenchymatous tissue, such as the liver, shows only a marginal lipogenesis on incubation of excised pieces (due to failure of the deeper cells to survive) whereas the other type of tissue, examplified by the cornea, shows uniform lipogenesis throughout the entire tissue.

The foregoing experimental observations would appear to have a direct bearing on the origin of fat in so-called fatty degeneration and possibly in atherosclerosis. Fatty degeneration consists in an accumulation of fat within viable cells on the edge of a necrotic lesion. Focal lesions of the liver induced *in vivo* show this rim of sudanophilia well. The fat forms promptly in the surviving cells but may be prevented from doing so by the addition of para-chloromercuribenzoate to the necrotizing agent.

A more direct visualization of lipogenesis from necrotic tissue may be obtained by injecting liver brei into the cornea. Fat will then form in the adjacent corneal cells similar to that which occurs when fatty acids exclusively are injected into the cornea. It will not occur if the lipid is first extracted from the liver brei. There would appear to be little doubt, therefore, that in this analogue of fatty degeneration, it was the liberated fatty acids of the necrotic liver which were inducing the lipogenesis in the corneal cells.

A similar process is believed to underlie the fat formation in atheromatosis, but crucial experiments to prove this have yet to be done. The question is, of course, why blood vessels and other tissue do not form fat abundantly during life, since it is obligatory for them to do so experimentally when exposed to native fatty acids and serum. A partial answer may be in the availability (or unavailability) of the fatty acids or, in other words, in the stability of the protein complexes that normally bind the fatty acids and may prevent their utilization by the cells of the body.

Of perhaps greater biologic significance than either fatty degeneration or atheromatosis is the usefulness of aberrant lipogenesis to the body economy. Free fatty acids are potent necrotizing agents and it would appear imperative to have a mechanism for promptly converting those which become available into an innocuous form. In the blood stream there is abundant evidence that physiologic quantities of fatty acids are bound promptly to albumin and thereby detoxified, but in the tissue the mechanism appears to be one of rapid esterification into a neutral and, therefore, innocuous triglyceride. In this form, it may either remain indefinitely as an inert fat reservoir or it

may be disposed of by the leisurely process of phagocytosis. According to this concept, which is admittedly speculative, aberrant lipogenesis serves a biologically useful function in protecting the body from one of its own, potentially toxic, constituents.

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"THERAPY" WITH VITAMIN E

Despite many claims to the contrary, convincing evidence is lacking that vitamin E is effective in treating threatened abortion or muscular dystrophy of unknown etiology.

A report, "Should Spontaneous Abortion Be Prevented," by E. V. Shute has appeared recently (*Canad. Med. Assn. J.* **82,** *72 (1960)*). As the title suggests, the article is biased. For many years Shute has published accounts of the therapeutic efficiency of vitamin E in such diverse conditions as pruritis vulvae, rheumatic fever, angina pectoris and myocardial infarction, infertility, threatened abortion, nephritis, and pre-eclampsia (A. V. Vogelsang and E. V. Shute, *Nature* **157,** *772 (1946)*; *Nutrition Reviews* **5,** *164 (1947)*; **7,** *118 (1949)*; Vogelsang, E. V. Shute and W. E. Shute, *Medical Record* **160,** *279 (1947)*; **161,** *155 (1948)*). Unfortunately, studies conducted by others have failed to confirm his claims.

A brief reappraisal of the history of vitamin E will aid in an understanding of the problem. An essential milk factor was studied in 1920 and found to be essential for the growth of animals (H. A. Mattill and R. E. Conklin, *J. Biol. Chem.* **44,** *137 (1920)*). Soon this substance was found to be necessary for reproductive processes as well as growth in animals. It was found in such vegetable foods as lettuce, peas, several cereals and particularly in wheat germ. It was also found in beef liver and in egg yolk (H. M. Evans and K. S. Bishop, *J. Am. Med. Assn.* **81,** *889 (1923)*). At the same time, scientific curiosity in the potential curative effects of other vitamins was growing rapidly and soon a number of reports appeared relating to vitamin E. The active substance was found to be a tocopherol which later was divided into three fractions: alpha, beta and gamma, having a relative activity in that order. An interesting chemical effect of these fractions was the protection of other fats from oxidation, but here their relative effectiveness was in reverse order (gamma, beta, alpha).

Meanwhile deficient states were induced in many species of animals (Evans and G. O. Burr, *J. Biol. Chem.* **76,** *273 (1928)*; H. S. Olcott, *J. Nutrition* **15,** *221 (1938)*; C. G. Mackenzie and E. V. McCollum, *Ibid.* **19,** *345 (1940)*), and others. Lack of this vitamin resulted in such abnormalities as a muscular dystrophy with creatinuria, peculiar lesions of the central nervous system, cardiac failure, pulmonary hemorrhage, testicular degeneration and absorption of gestational products in rats. Pathologic studies of muscular lesions disclosed a peculiar type of myositis which was accompanied by ceroid deposits in many instances (K. E. Mason and A. F. Emmel, *Anat. Record* **92,** *33 (1945)*). Muscle tissue from deficient animals was found to have an abnormally high rate of oxygen consumption (O. B. Houchin and Mattill, *J. Biol. Chem.* **146,** *309, 313 (1942)*), a finding which led to the observation that the vitamin has an anti-oxidation effect.

These and many other examples of ac-

curate investigation gave ample reason for clinical investigators to consider the possibility that vitamin E might have some curative or beneficial effect in human disease which resembled, even slightly, those induced in animals. Unfortunately uncritical evaluation of results led to a few initial reports which were unjustifiably enthusiastic. In every instance of scientifically planned and controlled studies the results of therapy with vitamin E have shown no significant effect (except in the rare instance of certain types of biliary disease).

In this most recent report, Shute (*loc. cit.*) describes the results of the administration of alpha-tocopherol (exact dose not stated) to 232 women who had "threatened abortion . . . (or) threatened miscarriages." No evidence is given for classifying these pregnant women in the above categories, but 195 (84 per cent) delivered children. Thirteen of these had congenital anomalies. There were no control subjects for comparison, and no other forms of treatment were tried. Thus there would seem to be no reason to believe that the medication had any effect.

A somewhat more objective report was described by G. M. Berneske, A. R. C. Butson, E. N. Gauld and D. Levy (*Canad. Med. Assn. J.* **82,** *418 (1960)*), who reported their observations of 33 patients with muscular dystrophy. The group included 16 boys with pseudohypertrophic juvenile dystrophy, one girl with a non-hypertrophic juvenile dystrophy, and 16 patients of both sexes with a facio-scapulo-humeral or limb-girdle type of muscular dystrophy. The patients ranged from six to 58 years of age and had been observed for significant periods of time prior to the study. Treatment consisted of the oral administration of 2000 international units of alpha-tocopherol succinate to 22 of the patients and a suitable placebo to the other 11. Careful records were made of the strength of various muscle groups. At the close of the study when the patients had been given these medications for periods of 11 months to three years, muscular strength was again evaluated and recorded.

The data indicated that there was no difference between the effect of alpha-tocopherol and that of the placebo. The experimental design, although imperfect, did serve to demonstrate this lack of effect. The study would have been of more value if the vitamin and placebo had been given to the patients according to the double blindfold technique and if the groups receiving each of these had been equal. Similarly, it would have been preferable to have given the treatment for the same length of time to each patient. Also it would have been helpful to have studied the urinary excretion of creatinine, but the negative effects seem sufficiently evident. Unfortunately, the authors made the statement that the results were "sufficiently indecisive, so that we feel that we should not draw dogmatic conclusions."

There can be no doubt that vitamin E is an essential in the human economy and instances of true deficiency have been reported. One such patient with xanthomatous biliary cirrhosis was described by C. W. Woodruff (*Am. J. Clin. Nutrition* **4,** *597 (1956)*). Similar deficiency was reported by H. M. Nitowsky, H. H. Gordon and J. T. Tildon (*Bull. Johns Hopkins Hosp.* **98,** *361 (1956)*). In these instances, infants who were suffering from cystic pancreatitis and associated congenital biliary atresia had low serum levels of tocopherol and had creatinuria. A recently described test evaluating the susceptibility of erythrocytes to hemolysis by hydrogen peroxide (C. S. Rose and P. György, *Am. J. Physiol.* **168,** *414 (1952)*) allows the investigator to detect more subtle degrees of vitamin E deficiency.

With the abundance of information available as to the chemical nature and the several functions of this vitamin and with knowledge of methods for detecting its concentration in the serum (its protection of erythrocytes

from undue hemolysis and its effect upon the urinary excretion of creatinine), it is surprising to find that clinical investigators persist in the unworthy method characterized by administration of a substance to patients with a given disease and willingness to ascribe any coincidental change to an effect of that substance. Equally regrettable is the willingness of editors of medical publications to accept uncritical reporting for publication. This has led to general discredit of vitamin E as well as to the necessity for frequent studies which result in negative reports.

HEPATIC COMA

Neurological symptoms accompanying severe liver disease may be controlled by emptying the colon, feeding a protein-free diet, and giving antibiotics to prevent putrefaction.

In patients suffering from advanced hepatic disease, neurologic disorders are common. These take the form of mental confusion, flapping tremor, impaired consciousness, coma and death.

Many attempts have been made to explain this syndrome commonly called hepatic coma. Experiments in dogs have demonstrated that shunting of the portal venous blood into the inferior vena cava (Eck fistula) results in an inability of the animals to tolerate much dietary protein. When fed meat, they develop strange behavior and may become comatose. Apparently bypassing the liver prevents normal metabolism of amino acids. Both experimental animals with an Eck fistula and cirrhotic patients have abnormal concentrations of ammonia in their blood after eating protein, although the correlation between levels of ammonia and state of consciousness has been imperfect. Most investigators, however, feel it is an important factor in production of hepatic coma.

Efforts have been made to decrease the quantity of ammonia in the blood. Glutamic acid promotes utilization of ammonia in the brain (*Nutrition Reviews* **15,** *314* (*1957*)), and arginine participates in the urea cycle whereby carbon dioxide and ammonia are converted into urea. Another amino acid, methionine, has been found to induce neurologic symptoms in patients with hepatic cirrhosis (*Ibid.* **14,** *336* (*1956*)), although the mechanism is not clear.

Attempts at treatment of this disorder have been partially successful and restriction of dietary protein seems to benefit many patients (*Nutrition Reviews* **14,** *74* (*1956*)). Another method depends upon oral administration of antibiotics (tetracycline or neomycin) to lessen the degree of intestinal putrefaction of food (A. M. Dawson, J. McLaren and S. Sherlock, *Lancet* **II,** *1263* (*1957*)). Although most clinicians have abandoned the use of glutamic acid or arginine, a few continue to find the latter helpful (J. L. Fahey, D. Nathans and D. Rairigh, *Am. J. Med.* **23,** *860* (*1957*)).

G. C. Willis has reported his experiences in managing 120 cases of hepatic coma or impending coma (*Canad. Med. Assn. J.* **82,** *191* (*1960*)). All of these had demonstrable liver disease; 100 had cirrhosis, 11 had acute hepatic necrosis, and nine had hepatic malignancy. By way of comparison, the author studied 100 other patients with cirrhosis who did not have coma or mental symptoms.

Of the 120 patients, 88 had flapping tremor, 86 had actual coma, and 80 were visibly jaundiced. Fetor hepatitis was noted in 28 per cent of the group with coma and in 25 per cent of those without. Factors which might have precipitated the onset of coma included gastrointestinal hemorrhage,

pneumonia, abdominal paracentesis and sedative drugs.

Treatment was as follows: correction of the precipitating factors, administration of magnesium sulfate and an enema, feeding a protein-free diet of 2000 colories, and oral administration of 250 to 500 mg. tetracycline every six hours. Seventy-eight patients survived. The prognosis was best in those with cirrhosis (74 per cent) and poorest in those with acute hepatic necrosis (9 per cent).

The author explains his reasons for believing that hepatic coma is caused by parenchymal cellular damage rather than by portal hypertension with secondary anastamoses. In the 100 cirrhotic patients without coma, the incidence of visible collateral circulation was greater (27) than in those with coma (25). Similarly, splenomegaly and ascites (additional signs of portal hypertension) were less common in cirrhotic patients with coma than in those without. The poor prognosis in those patients with acute hepatic disease would support his contention. Fetor hepaticus was thought to result from products of intestinal putrefaction and was observerd only once in the control group, but 25 times in those with coma.

Of the factors which may precipitate coma, gastrointestinal hemorrhage was most important. This might be due to hypotension, which reduced hepatic circulation, and to the increased putrefaction of blood. Ingestion of blood results in a greater rise of blood ammonia than ingestion of other proteins in like amount (A. N. Bessman and G. S. Mirick, *J. Clin. Invest.* **37,** *990 (1958)*).

Neither the exact cause of hepatic coma nor the ideal form of treatment has been found. The patient with this condition has many coexisting biochemical defects. Perhaps the abnormal concentration of ammonia in the blood, the loss of potassium from cells, as well as the toxic effect of products of intestinal putrefaction are all important factors in the production of this syndrome. S. Sherlock has employed a form of therapy similar to that of Willis (Sherlock, W. Summerskill, and A. Dawson, *Lancet* **II,** *689 (1956)*), but she used neomycin and, in addition, gave hydrocortisone to patients with acute hepatic necrosis.

Without the accouterments of a research grant, a biochemical laboratory or even the use of statistics, Willis has made a significant contribution by simply observing his patients and recording what he saw.

AMINO ACIDS IN PLASMA AND URINE IN KWASHIORKOR

Children with kwashiorkor were found to have an aminoaciduria. Also ethanolamine and beta-aminoisobutyric acid were found in the urine. The latter two substances disappeared upon feeding an adequate diet.

Kwashiorkor is a disorder caused by decreased protein intake. This means that the limiting nutrients are specific amino acids. It has been reported that better nitrogen balance occurs in individuals with kwashiorkor when they receive a mixture of 18 amino acids (including all the essential ones) than when they receive just the essential amino acids alone (*Nutrition Reviews* **15,** *132 (1957)*). Recently, J. C. Edozien, E. J. Phillips and W. R. F. Collis (*Lancet I, 615 (1960)*) have studied the free amino acids in plasma and urine of children with kwashiorkor.

These investigators observed 50 new untreated cases of kwashiorkor in children between the ages of one and four years. On admission they were kept on glucose and water with added potassium until the following day when the fasting blood and urine specimens were obtained. After these initial samples were taken, 41 of the children were

treated with a high-protein milk diet either as out-patients or in the hospital. The nine remaining individuals, none of whom was seriously ill or in evident danger, were studied on different regimens. Three of the children were fed for four days a diet high in carbohydrate but low in protein (calculated to contain less than five g. of protein per day). The other six were given identical diets to which was added 2 g. of L-methionine per day. At the end of this four-day period both groups were then placed on a high-protein diet. For controls, the investigators used 25 children of the same age range.

The authors found the average fasting plasma amino acid nitrogen level for healthy children to be 5.2 mg. per 100 ml. (4.1 to 8.0 mg.) if the method of Folin was used, and 4.3 (3.5 to 6.1) with the ninhydrin procedure. For patients with kwashiorkor, the plasma amino acid nitrogen levels were uniformly lower, being 2.5 mg. per 100 ml. (2.0 to 4.5) and 2.1 mg. per 100 ml. (1.3 to 3.5 mg.) by the two methods, respectively. Thus the plasma level of amino acid nitrogen appeared to be about 45 per cent less than that found in comparably healthy children of this area. A decrease in intensity of ninhydrin reacting spots appeared in the plasma of the patients with kwashiorkor, although this was not a uniform reaction. The amino acids which appeared to be most affected were cystine, methionine, valine, tryptophan, leucine, isoleucine and phenylalanine, although these procedures were only qualitative. In the chromatograms obtained from plasma of normal individuals ethanolamine could not be detected, but was clearly visible in the plasma obtained from 75 per cent of the patients with kwashiorkor. Also beta-aminoisobutyric acid could not be found in the blood of the controls, but was found in 30 per cent of the children with kwashiorkor.

In order to study the amino acid content of urine, these investigators used uniformly 50 microliters of urine without regard for volume or period of collection. Thus the excretion values are at most only semiquantitative results, and offer no data on the total daily excretion of amino acids. Ethanolamine was not found in any of the normal urines but beta-aminoisobutyric acid was detected in 8 per cent of the urine samples obtained from normal individuals. However, ethanolamine was excreted in the urine of 80 per cent of the patients with kwashiorkor and beta-aminoisobutyric acid was identified in the urine in every case. In many of the pathological urines this amino acid produced a dominant spot. It should also be noted that the essential amino acids could be identified only rarely on the chromatograms obtained from the urine of the patients with kwashiorkor; most of the amino acids present were the nonessential acids.

On adding methionine to the diet of the patients with kwashiorkor, there was a marked increase in all plasma amino acids. In three individuals in whom ethanolamine was initially present in the plasma, the ethanolamine disappeared after methionine supplementation. Although the ethanolamine in the urine was greatly diminished in all six cases, the amount of beta-aminoisobutyric acid was not affected by the administration of methionine.

These investigators believe that the aminoaciduria in kwashiorkor is of the renal type since there is increased excretion of amino acids in spite of the level of these compounds in the plasma. Although urine analysis rarely shows any specific urine abnormality and no specific lesion in the kidney has been described, these investigators believe that the renal tubular cells are suffering from a generalized loss of cellular contents including, among other proteins, specific cellular enzymes. As such, the aminoaciduria could be the result of a defect in the reabsorption mechanism of amino acids by the renal tubules.

The beta-aminoisobutyric acid probably has thymine as its precursor. It is believed that the large amounts of this unusual amino

acid may be derived from cellular desoxyribonucleic acid, which is released by the degradation of skeletal muscle and organ tissues. While ethanolamine has been known to be excreted with other amino acids in cases of liver disease, these investigators could find no constant relationship between the plasma ethanolamine and ethanolamineuria on the one hand and the results of various liver function tests on the other, although again it should be noted that the urine collections were not standardized periods. The high level of ethanolamine in plasma may be due to a metabolic block in which ethanolamine cannot be utilized in other synthetic processes.

While the generalized nutritional deficiency in kwashiorkor is well known to be a protein defect, studies of a type presented by these investigators provide information which later may reveal the mechanisms by which a protein deficiency produces such a wide spectrum of changes.

REFRACTORY RICKETS AS A SEX-LINKED CONGENITAL ANOMALY

Rickets resistant to vitamin D therapy appears to be a sex-linked dominant familial hypophosphatemia. Affected males show more intense lesions than females, but only daughters of affected males show hypophosphatemia.

The occurrence of rickets resistant to vitamin D therapy has been recognized (F. Albright, A. M. Butler and E. Bloomberg, *Am. J. Dis. Child.* **54,** *529 (1937)*), and the use of massive doses of vitamin D has been necessary to produce healing of the bone lesions in such instances. It has been suggested that the type of rickets which is refractory to dietary supplements of vitamin D results from a congenital or acquired metabolic defect.

J. B. Graham, V. W. McFalls and R. W. Winters (*Am. J. Human Genetics* **11,** *311 (1959)*) have recently studied large enough groups of related individuals to conclude that this condition is not a rare and exotic entity. These authors have characterized this condition as a sex-linked dominant familial hypophosphatemia, and their study of the clinical and chemical conditions associated with this disease represents an extension of an earlier report on a different family group (Winters *et al.*, *Medicine* **37,** *97 (1958)*).

Study of each instance of refractory rickets (as evidenced by bow legs, knock-knees, or the onset and progression of characteristic skeletal deformities despite vitamin D therapy, or by the typical radiographic signs of rickets in children or post-rachitic deformities in adults) led to identification of a kindred type exhibiting familial hypophosphatemia.

Hypophosphatemia was described by J. C. Rathbun as a developmental anomaly (*Am. J. Dis. Child.* **75,** *822 (1948)*), and Graham, McFalls and Winters (*loc. cit.*) have pointed out that it is only on the chemical evidence of low serum inorganic phosphorus that a genetic relationship can be detected. In order to detect abnormal serum inorganic phosphorus values in children, it was necessary to set up normal ranges by fitting regression curves by age and sex for a large normal population and using the 99 per cent confidence limit as a dividing point. Blood serum inorganic phosphorus values which fell below this limit were considered to be hypophosphatemic.

It was quite evident that bone deformities were more likely to occur in hypophosphatemic males than in affected females. On the other hand, daughters of affected men were uniformly hypophosphatemic while in no case did the son of an affected man have the condition. In the case of affected females, their progeny approached a ratio of 1:1:1:1 (affected males: normal

males: affected females: normal females), which would be expected of a sex-linked dominant character.

The problem of penetrance was considered by Graham, McFalls and Winters and they concluded that the hypophosphatemia is almost, if not completely, penetrant. The bone disease is far less so and is affected by a sex difference in the expression of the abnormal gene. Of males showing hypophosphatemia, 93 per cent showed bone disease, but only 25 per cent of the females exhibited bone changes. In addition, the severity of the bone lesions appeared to be more intense in males, with females having only moderately severe lesions.

These investigators have considered at some length the possibility that males may have a more depressed serum inorganic phosphorus value than females. Certainly the values presented indicated a mean value lower by 0.46 mg. per cent for the males. However, a careful evaluation of the values and associated factors of age and environment suggest that perhaps this difference should be viewed with caution until more complete studies of larger numbers of individuals can be made.

With respect to nutrition, it is of interest that the group involved in this study were predominantly from farm families who customarily consumed large quantities of dairy products and were generously exposed to sunlight. In practically every instance, individuals who showed signs of rickets as young children were given generous supplements of cod-liver oil. A low serum inorganic phosphorus value was consistently observed. This blood abnormality, however, was not consistently related to bone deformities, especially in females.

The observations of Graham, McFalls and Winters certainly emphasize the genetic basis for a rather common occurrence of refractory rickets. The almost complete elimination of dietary rickets from the United States should suggest that occurrence of rickets resistant to vitamin D therapy ought to be viewed as a possible congenital anomaly.

CARBOHYDRATE METABOLISM DURING ETHER ANESTHESIA

Without surgical trauma, ether anesthesia alone will induce changes in glucose metabolism as indicated by decreased glycose tolerance, increased inorganic phosphorus levels and changes in pyruvate concentrations of the blood.

The metabolic effects of trauma have been reviewed earlier (*Nutrition Reviews* **16,** *196 (1958)*). However, in studying the changes induced in patients following surgical trauma, it is necessary to separate the effects due to the surgical procedure from those caused by the anesthetic used in the operation. Ether (diethyl ether) is one of the common anesthetic agents used in surgical procedures and is usually used in conjunction with other substances such as nitrous oxide and barbiturates (thiopental sodium).

Knowledge that ether anesthesia induces metabolic changes in man is not new. A. Reynoso (*Compt. Rend. Soc. Biol.* **5,** *116 (1853)*) noted sugar in the urine of patients who were anesthetized with ether. This was less than ten years after the discovery of ether anesthesia. Over a period of years it has been frequently reconfirmed that ether anesthesia induced an elevated blood sugar level and frequently glycosuria.

Recently, a team of investigators led by W. R. Drucker (*Metabolism* **8,** *827 (1959)*) studied the effects of ether anesthesia on glucose and fructose metabolism by performing three intravenous glucose tolerance tests on each of six patients and three intravenous fructose tolerance tests on each of another six patients. The first (control) test was performed a day or two before the patient was anesthetized. The second test was

performed on the day of anesthesia including the period when the ether was being given, and the third test on the day following the anesthesia. No operations were performed at this time and the period of anesthesia was 90 minutes. The volunteers used in this study had been hospitalized for the rehabilitation of a nonprogressive illness several weeks before the study was undertaken.

The hexose tolerance tests were carried out by giving 1 g. of either glucose or fructose per kilogram of body weight per hour, in the form of a 10 per cent sugar solution. All infusions lasted one hour. Samples of venous blood were drawn at the beginning of each study and at 30-minute intervals for a total of three hours, and the levels of glucose, fructose, pyruvate, lactate and inorganic phosphorus were determined.

Such studies require a high degree of control if one wishes to eliminate numerous extraneous factors which would mask or confuse the metabolic aspects of anesthesia. These investigators believed that they incorporated three important features which increased the degree of control. The first was a fully equipped operating room in which to carry out all the studies. Thus familiarity with the environment tended to reduce anxiety on the day of anesthesia. Secondly, the anesthesia was given to all of the subjects by the same trained anesthetist. This eliminated variation in anesthetic procedures. Finally, no surgery was performed and thus the metabolic effects of anesthesia were not complicated by the changes induced by surgery.

Anesthesia alone caused a rise in the blood glucose levels along with an increase in lactate and inorganic phosphorus concentrations in the serum. The amount of fructose and pyruvate present in the blood did not change when ether was given.

When glucose tolerance tests were carried out on patients receiving ether, the glucose levels of the blood were elevated as compared to the control period of study. However, the decay rate of the glucose tolerance curve did not differ from that observed when the patient received no ether. No changes in the fructose tolerance curves were noted upon administering ether. However, there was an increased level of glucose in the blood following the fructose administration. Thus when glucose is formed from fructose in the body it cannot be utilized as well as the original fructose in the anesthetized patient.

A small rise in the pyruvate levels in the blood was noted after giving glucose, and a greater rise occurred if the patient was anesthetized. Fructose without anesthesia induced blood levels of pyruvate which were much higher than in the anesthetized patients receiving glucose. However, on giving ether to patients receiving fructose, smaller rises in the pyruvate levels were noted when compared to the non-anesthetized individual. On the day following ether, the fructose tolerance test caused a rebound in the pyruvate levels resulting in greater values than during the control period.

It is well known that during either a glucose or fructose tolerance test in a non-anesthetized individual there is a fall in the level of inorganic phosphorus in the blood, indicating that organic phosphorus compounds are being formed. However, in the present study in the individuals anesthetized with ether there was an initial rise in the inorganic phosphorus levels, which continued despite the administration of glucose. On the other hand, when fructose was given to an anesthetized patient this early rise was reversed and there was instead a decrease in inorganic phosphorus during the first 30 minutes of sugar infusion. Thereafter, however, the inorganic phosphorus level increased abruptly despite continued fructose administration. The initial fall in inorganic phosphorus on giving fructose intravenously also supports the view that fructose can be utilized by the anesthetized individual.

It has been thought previously that the changes in carbohydrate metabolism noted in the anesthetized patient are the result of

the secondary effects of increased adrenalin production induced by the ether. This group of investigators studied two individuals who were given sufficient adrenalin to cause their blood sugar to reach levels noted in patients given ether, and only a small rise in the blood pyruvate occurred along with a fall in the serum inorganic phosphorus levels. Also a series of studies was carried out with a patient who had a transection of the spinal cord high enough to preclude all central nervous stimulation to the adrenal medulla. On administering ether to this patient, no change in the pyruvate or rise in the inorganic phosphorus level was noted. Thus these two studies tend to rule out increased adrenalin production as the only causative agent in the metabolic changes induced by ether anesthesia.

On the day following the anesthesia, all of the metabolic changes that had been noted in carbohydrate metabolism disappeared. This is in contrast to the changes in carbohydrate tolerance observed in postoperative patients, which may last many days. The changes found following ether anesthesia are consistent with changes noted in a steroid type of diabetes, but the relationship between these two phenomena are not known. A fuller explanation of the cause of the abnormality in carbohydrate metabolism induced by ether is not yet available.

ACCUMULATION OF INSECTICIDES IN TISSUES AND EXCRETION IN MILK

Steers, swine, poultry and lambs show marked differences in the tissue accumulation of dieldrin. Both tissue accumulation and milk excretion levels are roughly proportional to the level in the feed.

Milk and milk products make up such a large proportion of the diet of children and adults in the United States that any change in milk composition through changes in agricultural practice or processing techniques is of great interest to everyone. The use of modern insecticides to protect plants and animals from harmful insects led inevitably to studies of the accumulation of insecticide residues in animal tissues used for food and the excretion of these residues in milk.

It was early demonstrated that the chlorinated hydrocarbon insecticides were stored in animal fat in varying degrees. Consequently, the extent to which these insecticides may be used on forage crops or on animals depends greatly on their propensity for storage in the tissues of animals and upon the excretion of these insecticides in milk. Some insecticides have been assigned tolerance levels in the fat of certain animals, but, at the present time, regulatory agencies have maintained that milk is not to be contaminated by pesticides or other foreign substances.

N. Gannon, R. P. Link and G. C. Decker (*J. Agr. Food Chem.* **7**, *824, 826, 829 (1959)*) in a series of three papers have investigated the storage of dieldrin in the tissues of steers, hogs, lambs, poultry and eggs, as well as the excretion of a number of insecticides (aldrin, dieldrin, D.D.T., heptachlor and methoxychlor) in milk of dairy cows fed these substances in the daily ration. Their results indicate that there are great differences in the way the different species handle dieldrin, but it is plain that the fat tissues accumulated dieldrin at all of the levels fed (0.1, 0.25, 0.75, and 2.25 p.p.m.). At the end of 12 weeks, the accumulation of dieldrin in the fat of the different species was roughly proportional to the level of insecticide in the diet.

Gannon, Link and Decker took great care to insure a quantitative intake of the

insecticide and, following a feeding period of 12 weeks, some of the animals and poultry were sacrificed to determine insecticide accumulation in the tissues. The remaining animals were retained on an insecticide-free diet to determine the rate of elimination over a period of six weeks. Their results demonstrate that tissue accumulation of dieldrin is directly related to the amount of fat in the diet.

There were distinct differences between species; steers stored about twice as much dieldrin as hogs and about five times as much as lambs. On the other hand, hens stored about ten times as much in their body fat as did steers, although they excreted a barely measurable amount in their eggs. In general, renal fat contained most insecticide, slightly exceeding the values obtained for body fat. This probably reflected the higher actual fat content of the renal fat tissues. In steers, roasts and steaks had the next highest storage values, although no dieldrin was measurable in these tissues when fed at levels of 0.25 p.p.m. Liver and kidney tissues stored the smallest amounts of insecticide.

Lambs showed no dieldrin in the chops over the entire range of dosages, and the level in the roast was probably not significant. Six weeks after dieldrin was discontinued, the fat of steers and hogs had dropped about 40 per cent while the fat of lambs had dropped about 70 per cent from the level attained after 12 weeks of feeding.

In dairy cattle, the level of dieldrin excreted in the milk was proportional to the level in the feed. When the cows were on a continuous intake of the insecticide, there appeared to be an approximate straightline relationship between p.p.m. of dieldrin in the milk and p.p.m. in the feed. By the end of the 12-week feeding period, dieldrin was present in the milk in measurable quantities at all levels of the insecticide feeding. As in beef cattle, dairy cattle tissues analyzed for dieldrin at the end of 12 weeks' feeding showed accumulations of dieldrin roughly proportional to the amount of fat in the tissues and to the level of insecticide in the diet.

The work of Gannon, Link and Decker suggests that the ratio of dieldrin in milk to the dieldrin in the fat of the tissues when the cattle are on a daily dieldrin intake will range between 12 and 18 to 1. After six weeks on a dieldrin-free diet, it was evident that the dieldrin in milk had dropped by 80 per cent while that in the body fat had dropped by 30 per cent from the levels attained at the end of the insecticide feeding period.

Residues in milk resulting from feeding other chlorinated hydrocarbon insecticides, as well as dieldrin, were also determined. The levels fed were as follows: aldrin 1, 10, 40 p.p.m.; dieldrin 10, 50, 75 p.p.m.; heptachlor 50, 75, 100, 200 p.p.m.; D.D.T. 10, 25, 100, 200 p.p.m.; and methoxychlor 800, 1000, 4000, 7000 p.p.m. The results of these trials indicated that aldrin (excreted as dieldrin) was excreted roughly twice as readily as dieldrin itself, ten times as readily as D.D.T., 20 times as readily as heptachlor (excreted as heptachlor epoxide), and 1600 times as readily as methoxychlor. Storage in the body fat was of the same order of magnitude as secretion in the milk.

While it was apparent that there was some difference in rate of loss of the insecticides as compared to rate of storage when evaluated over a period of seven and one-half weeks following discontinuation of insecticides, methoxychlor was lost most rapidly and D.D.T. was lost much more rapidly than the remaining insecticides studied.

The results presented by these investigators in this series of three papers are of great interest to both the consumer and producer of meat, milk and eggs, since they emphasize

the different ways in which various species metabolize and handle a given insecticide. Certainly such work will provide a firmer basis for evaluating the use of insecticides and establishing tolerance levels when these are used in animal feeds.

WATER-SOLUBLE AND -INSOLUBLE GROWTH FACTORS IN DIETS CONTAINING TUMOR TISSUE

The sodium ion content of tumor diets is responsible in part for increased food and water ingestion and carcass hydration in rats, but still other appetite and growth factors remain unidentified.

Previous studies (*Nutrition Reviews* **17,** *274 (1959)*) have demonstrated that lyophilized tumor tissue used as a source of nitrogen in the diet can stimulate food intake and weight gain in tumor-bearing Sprague-Dawley rats that have reached a weight plateau on a 20 per cent casein diet. The details of experiments pointing to sodium ion as an agent responsible for this phenomenon but suggesting the presence of an additional water-insoluble factor in tumor tissue have been published by J. White, J. N. Toal, F. K. Millar, and R. H. Brooks (*J. Nat. Cancer Inst.* **24,** *197 (1960)*).

The tumor diet contained Walker carcinosarcoma 256 tumor tissue, which had been lyophilized and defatted by treating the lyophilized tumor tissue with 2:1 alcohol-ether followed by 1:1 chloroform-methanol. Additional alcohol-ether removed the residual chloroform. The lyophilized defatted tissue was extracted five times with boiling distilled water for periods of 20 minutes each with the use of 3.75, 1.5, 1.5, 1.5 and 1.0 liters per kg. for successive extractions. The residue was washed with alcohol and ether consecutively and air-dried. The water-insoluble product (H_2O-I) contained 13 per cent nitrogen and 3 per cent ash and represented about 80 per cent of the defatted tissue. The water extracts, combined and lyophilized, resulted in a water-soluble product (H_2O-S), a nonhygroscopic, easily powdered, yellow solid containing 11 per cent nitrogen and 22 per cent ash.

These tumor fractions, H_2O-I and H_2O-S, and certain other known compounds tested (sodium chloride, potassium chloride, calcium acetate, calcium citrate, magnesium citrate, magnesium carbonate, ammonium citrate, sodium citrate and sodium acetate) were incorporated into diets or fed as supplements to a 20 per cent casein diet, C-20. Both the C-20 diet and a diet (T-24) containing 24 per cent lyophilized Walker 256 tumor tissue were used as standards for comparison. All diets were isocaloric and isonitrogenous, adjustments for differences in nitrogen being made by varying the quantity of starch in the diets.

The experimental diets were fed to Sprague-Dawley rats bearing the transplanted Walker 256 tumor, beginning 15 days after tumor transplantation. Two types of feeding procedures were used. In one procedure, all animals were fed the C-20 diet until they failed to gain weight, at which time they were given an experimental diet or supplement and the effect on changes on weight and food intake observed. In the second procedure, comparisons were made among groups of six to ten rats ingesting the experimental and control diets throughout the course of the experiment.

Food intakes and weights were measured daily. The animals were killed at the end of all experiments, their tumors and adrenals removed and weighed. In one experiment, carcasses (minus adrenals, livers and intestinal contents) and tumors were weighed, lyophilized and defatted with 3:1 alcohol-

ether followed by 1:1 chloroform methanol. The residues, washed with alcohol-ether, were lyophilized, the weight of the residue representing the fat-free dry weight. Fat content was calculated by difference.

Utilizing the first type of feeding procedure, the authors tested the water-insoluble fraction (H_2O-I) as a nitrogen source at the 22 per cent level (H_2O-I-22) and the water-soluble fraction (H_2O-S), added to the casein control diet C-20, at a concentration (4.5 per cent) comparable to its level in the whole tumor tissue diet, T-24. Diet H_2O-I-22, on replacing C-20 on the fourteenth day, failed to produce an increase in growth or food intake. However, addition of H_2O-S to diet C-20 produced an increase in weight parallel to that of animals fed diet T-24 throughout the experiment.

These findings suggested to the authors that a growth-stimulating factor resided in the water-soluble fraction and led them to purify further H_2O-S by putting an alcohol extract of that fraction through a charcoal column. The forerun yielded a product containing only 3 per cent nitrogen and having a high inorganic salt content (13 per cent sodium and 13 per cent potassium). The inorganic fraction, fed as a supplement to diet C-20, produced dramatic overnight weight gains in a rat, mostly due to an increased water intake.

Subsequent experiments, in which a variety of inorganic salts (see above) were fed as supplements (0.1 to 0.2 g. per day) to animals that had attained a plateau on the C-20 diet, suggested that the sodium ion was the specific cation responsible for the stimulating effect.

Utilizing the second type of feeding procedure, the authors compared the effects of additional dietary sodium chloride on the total body weight of tumor-bearing rats and on the weight change and composition of carcass and tumor at two levels (0.9 and 1.8 per cent of the diet) with the effects of diets C-20 and T-24. All diets contained initially a salt mixture (R. B. Hubbell, L. B. Mendel and A. J. Wakeman, *J. Nutrition* **14,** *273 (1937)*) supplying 0.3 per cent sodium chloride. Diet C-20 plus 0.9 per cent sodium chloride was equivalent in sodium content to diet T-24. When the four diets were fed ad libitum to rats bearing 15-day-old tumors, the two salt-containing diets were as effective as T-24 for the greater part of the experiment, *i.e.*, for 19 to 23 days. Increasing the sodium chloride content twofold had no increased effect. The total body weight, including tumor, and the weight of the tumor were greatest for the group on the T-24 diet, although animals receiving the high-salt diets had the greatest food intake.

In a separate experiment, tumor-bearing rats on the C-20 plus 1.8 per cent sodium chloride diet were pair-fed to a group ingesting T-24. Their gains in weight were less than those of rats on T-24 or C-20 fed ad libitum, indicating, in the author's opinion, that the growth rates of animals ingesting salt diets ad libitum (which matched the rates of animals ingesting T-24) could be attributed to increased food intake. These experiments led the authors to conclude that sodium chloride cannot duplicate tumor effects completely.

The adrenal weights of animals bearing large tumors and ingesting C-20 were greater by a factor of three than those of normal control animals. However, adrenal weights of animals ingesting T-24 or the high-salt diets were slightly less than twice the weights of the normal controls. The *zonae glomerulosae* in adrenals of animals ingesting the high-salt diets were appreciably narrower and had lower lipid contents, as judged from Oil Red 0 stain. The authors interpret these adrenal findings as indicating that salt prevents an adrenal hypertrophy in tumor-bearing animals, which, they believe, have an increased demand for salt.

Carcass and tumor composition of Walker 256 tumor-bearing rats ingesting the C-20, T-24 and C-20 plus 1.8 per cent sodium

chloride diets ad libitum were measured as described above in one experiment. On all three diets, the rats lost carcass weight as the tumors increased in size. Inspection of the carcass analysis data reveals that, on the C-20 diet, about 60 per cent of the carcass weight loss was lost as water, 17 per cent as fat-free dry weight, and 24 per cent as fat. On the T-24 diet, about 49 per cent of carcass weight loss was lost as water, 23 per cent as fat-free dry weight, and 28 per cent as fat. On the C-20 plus 1.8 per cent sodium chloride diet, 53 per cent of carcass weight loss was lost as water, 19 per cent as fat-free dry weight, and 28 per cent as fat. Figures for water as a percentage of fat-free wet weight were slightly higher for the T-24 and C-20 plus 1.8 per cent sodium chloride diets (76.2 and 74.7 per cent, respectively) than for the C-20 diet (73.0 per cent), but only slightly different from the control percentage (74.4). There was no apparent influence of diet on the water content of tumor. The authors believe these data are additional evidence for the presence of a disturbance in water and electrolyte metabolism in these tumor-bearing rats.

As evidence for a possible factor in the water-insoluble fraction (H_2O-I), the authors cite data obtained under conditions of prolonged H_2O-I feeding. When three groups of rats bearing 15-day tumors were fed on C-20, T-24 and H_2O-I-22, respectively, for 25 days, the animals on C-20 reached a weight plateau, those on T-24 continued to grow and those on H_2O-I-22 showed first a delay in growth but then a growth rate almost equal to that of animals on the T-24 diet. The delay was associated with a 15 to 25 per cent decrease in food intake. Rats fed H_2O-I-22 grew tumors equal to or larger than those fed T-24, and both groups had larger tumors than those fed C-20. However, carcass weight loss was greatest in animals fed H_2O-I-22. The possibility that the effect of H_2O-I-22 on tumor growth might be due to its content of sodium ion was ruled out by the low ash content (3 per cent) of the H_2O-I fraction. When the experiment was repeated with an additional group of rats ingesting H_2O-I-22 into which 0.9 per cent sodium chloride had been incorporated, the group receiving H_2O-I-22 plus added salt grew the largest tumors but showed less carcass weight loss than any group except that ingesting T-24.

This work demonstrates the necessity of isolating and testing biologically the individual components of so heterogeneous a tissue as Walker carcinosarcoma 256 if sense is to be made of the appetite and growth-stimulating effects of tumor-containing diets. The well-known effects of sodium ion on water metabolism and the failure of sodium ion supplementation of the C-20 diet to duplicate the effects of the T-24 diet over extended periods suggest more strongly than the authors indicate that the significant developments in this problem lie ahead. Experiments designed to eliminate the distractions produced by the sodium ion are indicated and reports of such studies, following a promised communication on electrolyte balance studies, may be anticipated.

LYSINE AS A CATION IN POTASSIUM DEFICIENCY

Lysine appears to function as a cation contributing to the maintenance of ion balance in the muscle cells of potassium-deficient animals.

The loss of potassium from skeletal muscle of rats fed a diet deficient in potassium is accompanied by an increase in intracellular sodium equivalent to one-half to two-thirds the amount of potassium lost (H. C. Miller and D. C. Darrow, *Am. J. Physiol.* **130,** *747 (1940)*; E. Muntwyler and G. E. Griffin, *J. Biol. Chem.* **193,** *563 (1951)*). Thus, muscle

of potassium-deficient animals is relatively deficient in alkali metal cations.

The following observations have suggested that the apparent deficit is compensated by an increase in intracellular hydrogen ions. (1) During recovery from potassium deficiency, the alkali cation deficit of muscle is corrected and rats lose hydrogen ions in an amount roughly equal to the deficit (R. E. Cooke *et al.*, *J. Clin. Invest.* **31,** *798 (1952)*). (2) L. I. Gardner *et al.* (*J. Gen. Physiol.* **36,** *153 (1952)*) found the intracellular pH of muscle from potassium-deficient animals (calculated from carbonic acid determinations) to be lower than that of muscle from control animals. Finally (3), J. Orloff *et al.* (*J. Clin. Invest.* **32,** *538 (1953)*) found that plasma pH decreased following injection of potassium chloride into nephrectomized potassium-deficient rats. No plasma acidification occurred when sodium chloride was injected.

An hypothesis that cations other than hydrogen may increase in the cells during potassium deficiency has been suggested by R. E. Eckel, J. E. C. Norris and C. E. Pope II (*Am. J. Physiol.* **193,** *644, 653 (1958)*). This possibility, that a portion of the potassium deficit might be made up by an increase in the concentration of cations not usually determined, was in part suggested by the work of H. N. Christensen *et al.* (*J. Biol. Chem.* **198,** *17 (1952)*), who found that 2,4-diaminobutyric acid competes with potassium for entrance into a type of tumor cell.

In their studies to test this hypothesis Eckel *et al.* (*loc. cit.*) produced potassium deficiency in rats either by simple dietary deficiency or by steroid administration; or, for very rapid production, by protein depletion followed by the feeding of a high-protein, potassium-free diet. Male rats initially weighing 100 to 140 g. were kept on the depletion regimen from 13 to 29 days. Sodium and potassium concentrations of muscle, plasma and urine were determined by flame photometry, chloride by electrometric titration, and lysine was measured quantitatively by microbiological assay. Total amino acids were measured by paper electrophoresis and colorimetric reaction with copper sulfate.

A cation which was found in increased amounts in muscles from potassium-deficient rats was identified as L-lysine by four methods. (1) Using paper electrophoresis, the behavior of the cation was compared at two pH values and in three different solvents with the behavior of lysine, arginine and ornithine. (2) The absorption spectrum of the reaction product of the cation with ninhydrin was determined. (3) The theoretical yield of carbon dioxide and appearance of cadaverine were obtained after the action of lysine decarboxylase. Finally (4), the growth of *Leuconostoc mesenteroides* P-60 on a lysine-free medium supplemented with the cation was measured.

Rats treated with desoxycorticosterone acetate grew normally. However, they developed hypokalemic alkalosis and lowered plasma and muscle potassium concentrations just as did rats fed the potassium-deficient diets, indicating that these changes are not responsible for the poor growth and failure of protein synthesis in the potassium-deficient rats.

The most interesting observation from the first study was the four to tenfold increase in lysine and in total basic amino acids in the muscle of potassium-deficient rats, regardless of the cause of the potassium deficiency. Plasma lysine was increased in dietary potassium deficiency but was not consistently changed by the other two treatments. In rats kept on a potassium-deficient diet for two weeks, increase in basic amino acids accounted for the whole alkali-metal cation deficit. However, in rats kept on potassium-deficient diets for four weeks, the basic amino acids accounted for only one-third of the deficit. There was no increase in acidic amino acids nor any other change in the muscle amino acids of the deficient rats. Although potassium-deficient rats, repleted by potassium chloride injections,

had slightly decreased muscle basic amino acids when compared to sodium chloride-injected rats, there was no increase in excretion of lysine or lysine metabolites.

The second study reported by Eckel *et al.* (*loc. cit.*) was designed to show whether lysine and potassium compete for entrance into the cell and whether lysine affects the renal excretion of potassium. Muscle electrolytes and amino acids of rats fed the control or the potassium-deficient diet, supplemented with 10 per cent of L-lysine as the hydrochloride, were determined. The effects on muscle constituents and urinary potassium of supplementing the deficient diet with lysine hydrochloride or with ammonium chloride were also determined. In both series, the group with the lower food consumption determined the amount of food fed to the other group.

The concentration of lysine in the muscles of control and potassium-deficient rats receiving a lysine supplement increased well above values for the comparable unsupplemented groups. Lysine supplementation did not decrease muscle potassium nor increase potassium excretion above that caused by ammonium chloride supplementation. Thus these observations demonstrate that accumulation of basic amino acids does not, in itself, cause loss of potassium from muscle, and indicate that lysine and potassium do not compete for entrance into the cell or for reabsorption or excretion by the kidney.

The observations that basic amino acids accumulate in the absence of potassium depletion, and that the concentrations of both potassium and basic amino acids in muscle decrease as the potassium depletion period is extended from 13 to 29 days, make it difficult to estimate the significance of the accumulation of basic amino acids in muscle. However, the observation that there is a decrease in muscle basic amino acids during potassium repletion (as reported in their first study) supports their hypothesis explaining the increased hydrogen ion content of urine during potassium repletion. According to their scheme, during repletion, lysine leaves the muscle cells accompanied by hydrogen ions and this results in the urinary excretion of 1 mole of hydrogen ions per mole of lysine extruded from the cell.

These studies by Eckel *et al.* indicate that changes which would cause intracellular acidosis, *i.e.*, deficiency of cations, weak bases or excess of anions, do not occur in the muscle of potassium-deficient rats. However, previous workers (L. I. Gardner *et al.*, *J. Gen. Physiol.* **36,** *153 (1952)*) have reported a decreased intracellular pH in potassium deficiency. Thus, in order to study the possibility of intracellular acidosis, Eckel, A. W. Botschner and D. H. Wood (*Am. J. Physiol.* **196,** *811 (1959)*) studied the buffering capacity, ionic equivalence by paper electrophoresis, organic acid content, and the pH of muscle by carbon dioxide determination and by direct measurement. Throughout the study, careful attention was given to possible methodological sources of error.

No differences between control and potassium-deficient muscle were revealed by these techniques. Different absolute values for pH (pH 7.1 *versus* 6.9) were obtained by the two methods, but differences between control and deficient muscles did not exceed 0.06 to 0.08 pH units. The pH and buffering capacity of potassium-deficient muscle was about the same as that of normal muscle.

These studies have demonstrated that, early in potassium deficiency, increased amounts of lysine are found intracellularly; that there is no evidence for an increase in intracellular hydrogen ion concentration during this time; and that lysine does not compete with potassium for entry into the cell. When rats remain on potassium-deficient diets for as long as four weeks, muscle lysine decreases. It is, therefore, possible that the characteristics of muscle from animals suffering a prolonged deficiency of potassium may differ from those described here, which were observed rela-

tively early during the development of potassium deficiency. The main point of interest for nutritionists is the possibility that lysine may function as a cation contributing to the maintenance of ion balance n muscle cells.

SCURVY AND BLOOD COAGULATION

The vascular abnormalities resulting from a deficiency of ascorbic acid include a change in capillary permeability, anemia, a reduction in platelets, an increase in fibrinogen and a reduction in the levels of some of the plasma components required for blood coagulation.

Petechial hemorrhage is a prominent symptom of ascorbic acid deficiency. It is generally accepted that the primary disturbance producing the petechiae is a change in the permeability of the capillaries. However, over the years there have been reports that there are additional changes in scorbutic individuals and animals involving the coagulability of the blood and its content of cellular components.

The most commonly recognized blood change in scurvy is the anemia (*Nutrition Reviews* **3,** *178 (1945)*). The anemia seen in adult scurvy responds promptly to ascorbic acid supplementation. It is still impossible, however, to determine the extent to which ascorbic acid increases the hemoglobin level by an action on the hemopoietic system or by facilitating the absorption of iron (*Ibid.* **13,** *165 (1955)*; **16,** *113 (1958)*).

There is less agreement as to the changes in blood coagulation associated with a deficiency of ascorbic acid. Although considerable work has been done on the coagulation defect in this deficiency state, there is considerable divergence of opinion as to the magnitude and cause of the alteration.

R. J. Salmon and C. D. May (*J. Nutrition* **46,** *515 (1952)*) found evidence in monkeys "that ascorbic acid plays a role in the production of plasma fibrinogen. . . ." In 1943, W. R. Sullivan, E. O. Gangstad and K. P. Link (*J. Biol. Chem.* **151,** *477 (1943)*) observed a prolongation in the prothrombin time of the plasma of guinea pigs from 28 seconds during the control period to 40 seconds when they developed scurvy. The prothrombin time increased to as much as 200 seconds after a 5 mg. dose of 3,3′-methylenebis-(4-hydroxycoumarin) and stayed that way for as much as two weeks. Normal guinea pigs receiving 10 mg. of the anticoagulant showed a prolongation of the clotting time to about 80 seconds with a return to normal in 48 hours (R. S. Overman *et al., J. Biol. Chem.* **142,** *941 (1942)*). The ability to restore normal prothrombin levels after the administration of an anticoagulant is markedly reduced.

A. K. Presnell (*J. Nutrition* **8,** *69 (1934)*) observed that the coagulation time of blood taken from an ear vein increased within three weeks after the guinea pigs were put on a scorbutogenic diet to 4.9 minutes compared to 2.7 for the controls.

Recently, P. Barkhan and A. N. Howard (*Brit. J. Nutrition* **13,** *389 (1959)*) studied the changes in blood constituents during the development of scurvy in guinea pigs. They fed 250 g. guinea pigs the purified diet developed by M. E. Reid and G. M. Briggs (*J. Nutrition* **51,** *341 (1953)*) until the animals attained a weight of 300 g. Then 13 of them were put on the diet from which the ascorbic acid had been removed. These animals continued to grow normally for ten or 12 days and then lost weight, with death occurring after 22 to 28 days. At the time of death, the guinea pigs weighed 190 g.

After being on the scorbutogenic diet for ten days, the five guinea pigs tested had a platelet count of $900 \pm 177 \times 10^{-3}$ per

cubic millimeter compared to 491 ± 39 in the controls. On the tenth day, the prothrombin time as measured by the one-stage technique was 28.2 ± 0.4 seconds compared to 26.1 ± 0.6 in the controls. By the nineteenth to the twenty-fourth day of the scorbutic regimen, the animals showed no further increase in platelets but a prolongation of the prothrombin time to 32.7 ± 1.5 seconds.

To determine the specific factors in blood which were responsible for the prolonged prothrombin time, Barkhan and Howard (*loc. cit.*) carried out a number of tests which provided presumptive evidence for deficiencies, especially of the thromboplastic components of blood and the plasma accelerators or co-factors essential for the initiation of thromboplastic activity.

At this point, it becomes necessary to digress for a moment in order to indicate that one of the more widely accepted theories of blood coagulation (R. Biggs and R. G. MacFarlane, *Human Blood Coagulation and its Disorders, second edition. Blackwell, Oxford (1957)*) is that thromboplastin is formed by the interaction of a number of blood components. Those that are most clearly defined are: (1) platelets, (2) antihemophilic globulin (AHG), and (3) the plasma thromboplastic component (PTC). One of the latter two components is missing from the blood of true hemophiliacs, and the two types of hemophilia are classified on the basis of which of these blood coagulation components is lacking. The three components react with calcium and other less clearly defined components to form thromboplastin. The less clearly defined components have been recognized from studies of various congenital hemorrhagic diseases; these include the plasma thromboplastic antecedent factor (PTA), the Stuart factor, Factor I and others.

The conversion of prothrombin to thrombin occurs under the influence of thromboplastin and is apparently accelerated by at least two other well-defined substances, Factor V and Factor VII. The blood level of the latter (Factor VII) is reduced together with that of prothrombin by dicoumarin-like compounds.

The studies of Barkhan and Howard indicated that there may be low blood levels of Factor VII as well as plasma and serum thromboplastic factors (PTA and PTC) in scurvy. Their conclusions are open to some question since there is no way of knowing whether the presumptive tests for deficiencies of coagulation factors found in human blood are applicable to animal blood. Some factors, particularly the accelerator, Factor VII, show marked species differences in properties and blood concentrations. Furthermore, the abnormalities observed by Barkhan and Howard were quite variable (they were seen in about 50 per cent of their guinea pigs) and involved the less clearly defined thromboplastic components of the coagulation scheme.

It is interesting that, regardless of the type of defect, it could not be corrected by an injection of ascorbic acid a few hours prior to withdrawing the blood sample.

The blood from the normal and scorbutic animals retracted equally well with the clots maintaining their integrity at 37°C for at least five hours. This indicated that there were no gross abnormalities in clot retraction and lysis in the blood of the scorbutic guinea pig. In addition, blood from the scorbutic and control guinea pigs gave similar results in the assays for Factor V.

Scurvy appears to influence the blood-vascular system in a number of ways. The abnormality in vascular permeability and the presence of anemia are well documented. There appears to be an abnormality of blood coagulation, an increase in blood fibrinogen and possibly also in platelet levels. Variable changes probably occur in some of the blood components involved in the coagulation process, but these changes are likely to be of no physiological significance.

Approaches have been made to identify the specific factors responsible for the blood

coagulation abnormality. The work done so far suggests that the thromboplastic factors and certain co-factors or accelerators of thromboplastic activity are involved. More work is required before a final decision can be made as to factors that are deficient.

Much of the older work with scorbutic animals is complicated by the presence of concomitant nutritional deficiencies. Today it is possible to produce an uncomplicated ascorbic acid deficiency in both guinea pigs and monkeys, and techniques have been perfected for the more precise assay of a variety of the constituents that are involved in blood coagulation. Here is a field awaiting the active collaboration of competent hematologists and nutritionists.

SATURATION OF DIETARY FATS AND LIVER AND SERUM LIPIDS

Thirteen edible fats were fed to rats during a post-weaning period of seven weeks. Liver and serum cholesterol values were determined as well as the distribution of unsaturated fatty acids in cholesterol esters.

It has been demonstrated that diets containing large amounts of unsaturated fatty acids promote the reduction of serum cholesterol levels (L. W. Kinsell *et al.*, *J. Clin. Nutrition* **1,** *224 (1953)*). This action may be the result of essential fatty acids facilitating the transport or catabolism of cholesterol, thus preventing its deposition in arterial walls.

The action of dietary linoleate may be catalytic and require only trace amounts. It could, on the other hand, require relatively large quantities so that considerable amounts of cholesterol could be esterified. The fact that therapeutic diets rich in unsaturated fat are high in calories may be a disadvantage. Accordingly, experiments which help to clarify the role of unsaturated fatty acids in cholesterol metabolism are valuable. Although much significance is attached to the level of circulating cholesterol, it does not necessarily indicate the amount of tissue cholesterol or its ease of mobilization.

Experiments have been reported by R. Okey *et al.* (*Metabolism* **8,** *241 (1959)*) in which the effect of unsaturation of dietary fats on liver and serum lipids has been studied. These experiments were designed to determine the extent to which the fatty acids of liver cholesterol esters were altered by feeding fats of differing compositions.

Each of 13 fats were added so as to make up 10 per cent of an adequate fat-free basal diet. Each of these diets was fed to a group of ten male and 10 female rats, while a second group of 20 rats received a 1 per cent cholesterol supplement added to each diet. The feeding began at weaning and lasted seven weeks. Tissue samples were extracted to determine total lipids and cholesterol, and cholesterol esters were isolated using silicic acid columns. After saponification and extraction of cholesterol, the fatty acids were acidified and extracted. Spectrophotometric analysis was used to determine the percentages of polyunsaturated fatty acids. As a further check, silicic acid paper chromatography was used. This gave information about the monoenoic acid content.

Assorted oils were used, including the following listed in order of decreasing iodine number: menhaden, safflower, corn, soy, cottonseed straight and cottonseed hardened, peanut, olive, lard, shortening, butter oil, butterfat and coconut. The per cent content of monoene, diene and polyene fatty acids was determined.

Weight gains of males averaged between 225 and 272 g. without cholesterol, and between 195 and 244 g. with cholesterol. Females without the cholesterol supplement averaged between 141 and 169 g. gained.

With cholesterol, the females averaged between 137 and 177 g. The efficiency, or gain per gram of diet eaten, was similar in all males, ranging from 0.32 to 0.40 g. In the females, the lowest gain per gram eaten was 0.25 g. and the highest was 0.34. There was no apparent relationship between diet content of unsaturated fatty acids and the amount of weight gain shown.

Liver weights ranged between 8.3 and 14.3 g. in males and between 5.2 to 8.2 g. in females.

Liver free cholesterol averages fell to between 0.16 and 0.26 g. per cent in both males and females without cholesterol. Values for males receiving cholesterol extended from 0.22 to 0.39 g. and, for females, from 0.25 to 0.41.

Serum cholesterol levels averaged from 53 to 84 mg. per cent in males and from 50 to 86 mg. per cent in females when no cholesterol was fed. When males were fed cholesterol, they averaged from 55 to 96 mg. per cent and, in females fed cholesterol, from 68 to 219 mg. per cent.

In discussing their experiments, Okey *et al.* point out that their diets did not contain enough fat to induce fatty livers. Their purpose was to measure the result of alteration in composition of dietary fat on the retention of food cholesterol. One trend observed in the young males was that liver cholesterol values decreased as iodine numbers decreased, but that serum cholesterol levels remained relatively constant. This same finding was reported by C. H. Best, C. C. Lucas, J. M. Patterson and J. G. Ridout (*Canad. J. Biochem. Physiol.* **36,** *613 (1958)*), although their experiments were not strictly comparable.

As the mean chain length of dietary fatty acids increased, the liver lipids and cholesterol levels tended to increase. The cholesterol liver storage observed in rats fed hardened cottonseed oil suggested that long-chain saturated fatty acids may have been influential. Cholesterol-fed females fed safflower seed oil, cottonseed oil, olive oil, lard, shortening and coconut oil had high serum cholesterol levels. Two possible causes were proposed by the authors. One is the solubility of cholesterol esters in the dietary fatty acids, and another, the possibility that some substance in the oils inhibited hepatic destruction of estrogen, thus allowing more effect on serum cholesterol. The males fed cholesterol had a lower level of circulating cholesterol and higher liver lipids than the females, with the exception that liver cholesterol was higher in the females when the rats were fed menhaden, safflower, corn and olive oil.

Circulating cholesterol is utilized in intermediary metabolism and restored from the liver reservoir, and the rat appears to be unharmed while efficiently storing large quantities of cholesterol in the liver. If this storage is pathological, then the data on growth and liver and serum cholesterol do not suggest that dietary essential fatty acids or linoleate are able to control the accumulation.

Analysis for unsaturated fatty acids of liver cholesterol esters appear to reflect the pattern of these compounds determined in the diet oils.

Oleic acid appeared to be a prominent constituent of liver cholesterol esters, even when dietary fat was not rich in oleate. Several questions are suggested in studying the data found in these experiments. Is the liver cholesterol oleate easily taken up by the blood? Does the fact that male animals have more liver cholesterol oleate than females have any bearing on the high serum cholesterol levels of females? What is the significance of the finding that females have a high serum cholesterol when fed coconut oil, which is essentially without linoleate to assist in cholesterol transport? Why does arachidonate, a tetraenoic acid, appear as a constituent of cholesterol esters in animals receiving a diet low in linoleate if linoleate is the only precursor of arachidonate?

The answers to these questions, as well as more detailed knowledge of the fatty acid spectrum of lipids associated with pathological states including arteriosclerosis, require further study. If one assumes, as is usually justifiable, that the serum cholesterol concentration gives the best index of whether or not conditions are favorable for the occurrence of atherosclerosis, then some of the serum cholesterol values obtained in these experiments are very difficult to interpret. There is already considerable experimental evidence suggesting that some of the fats which led to the relatively high serum cholesterol values in the females in this experiment are highly protective against fatty arterial disease in the rat.

It should be pointed out, however, that the results of this type of experiment might be quite different with a higher or lower protein intake or with older animals or with a different length of experimental feeding.

EFFECTS OF DIETARY FAT, INOSITOL, VITAMIN B_{12} AND GLUCOSE-CYCLO-ACETOACETATE ON COAGULATION

Essential fatty acids, inositol, vitamin B_{12} and glucose-cyclo-acetoacetate partially inhibited an increase in blood platelets, coagulability and various lipids of some tissues and sera of rabbits fed 20 per cent saturated peanut oil.

A controversy related to cardiovascular investigation concerns the possible effects of dietary fat on coagulation and thrombolysis (*Nutrition Reviews* **17,** *218, 263 (1959)*). Meals high in fat, particularly if the fat is saturated, have been reported to produce shortening of clotting time. This has been observed in dogs and humans after ingestion of cream (G. G. Duncan and J. M. Waldron, *Trans. Assoc. Am. Physicians* **62,** *179 (1949)*), in humans fed animal fat (H. W. Fullerton, W. J. A. Davie and G. Anastasopoulos, *Brit. Med. J.* **2,** *250 (1953)*), in man fed meals of saturated or unsaturated fat (A. Keys, R. Buzina, F. Grande and J. T. Anderson, *Circulation* **15,** *274 (1957)*), and in man after ingestion of butter (N. F. Maclagan and J. D. Billimoria, *Lancet* **II,** *235 (1956)*). Reduction of platelets and clotting time was observed in rabbits fed cream by R. L. Swank and C. F. Cullen (*Proc. Soc. Exp. Biol. Med.* **82,** *381 (1953)*). In addition, both *in vitro* and *in vivo* in animals and *in vitro* in man, saturated fats inhibit fibrinolysis activated by streptokinase (W. A. Thomas and R. F. Scott, *Ibid.* **96,** *24 (1957)*).

Similar findings have been reported by other groups (Fullerton, *Brit. Med. J.* **1,** *663 (1955)*; H. B. W. Grieg, *Ibid* **2,** *708 (1957)*; E. Sohar, M. C. Rosenthal and D. Adlersberg, *Am. J. Clin. Path.* **27,** *503 (1957)*). Some of these results may be relevant to the cholesterogenic and atherogenic effects of saturated fats under certain dietary conditions in animals (*Nutrition Reviews* **18,** *23 (1960)*). It has also been shown that saturated fats incorporated into rather unphysiologic food mixtures favor a high incidence of myocardial infarcts in rats (Thomas, W. S. Hartroft and R. M. O'Neal, *J. Nutrition* **69,** *325 (1960)*).

However, there are other reports in which such effects of dietary fat (animal or vegetable) have not been obtained respecting either coagulation or fibrinolysis (G. H. Hall, *Brit. Med. J.* **2,** *207 (1956)*; C. Merskey and H. L. Nossell, *Lancet* **I,** *806 (1957)*; S. I. Nitzberg *et al.*, *Circulation* **19,** *676 (1959)*). Nitzberg *et al.*, however, did find a shortening of Stypven time in hyperlipemic man and prolongation of profibrinolytic activity. A comprehensive review of dietary fats and blood coagulation recently published emphasizes much of the controversy (S. A. Hashim and R. E. Clancy, *New Engl. J. Med.* **259,** *1115 (1958)*).

A report by M. C. Nath and A. Saikia (*J. Nutrition* **69,** *403 (1959)*) may help elucidate aspects of this controversy. Their results lend support to the hypothesis that saturated fats fed to rabbits both enhance experimental atherosclerosis and favor coagulation (shortening bleeding time, coagulation time, prothrombin time). Their experiments differ somewhat from previously published ones in that they controlled the levels of essential fatty acids, inositol, vitamin B_{12} and glucose-cyclo-acetoacetate in their food mixtures (*Indian J. Med. Res.* **47,** *73 (1959)*).

The controls received native peanut oil (6 per cent), whereas the experimental rabbits received 20 per cent hydrogenated peanut oil. The remainder of the diet corresponded to semi-synthetic mixtures used in this country (casein 20 per cent, sucrose 30 per cent, wheat flour 39 per cent, salt mixture 5 per cent, and vitamin mixture 3 per cent, by weight). The experimental diets with the higher fat content were made isocaloric with the control at the expense of the carbohydrate. Additional experimental groups received 1 per cent linoleic acid, linolenic acid or inositol added to the saturated peanut oil. Other groups received, in addition to saturated fat, daily injections of either 15 micrograms of vitamin B_{12} per kg. body weight or 300 mg. per kg. body weight of hydrolyzed glucose-cyclo-acetoacetate.

After 12 weeks on the above regimens, the bleeding time, coagulation time, prothrombin time, blood platelet counts and hemoglobin contents of the blood were determined in the fasting animals. The animals were then sacrificed and total and esterified cholesterol levels as well as lipid phosphorus levels of the serum measured. Hepatic and cardiac lipids (total, free cholesterol, ester cholesterol, phospholipid) were estimated biochemically.

The authors reported statistically significant effects of feeding the high levels of dietary saturated fat on the bleeding time, coagulation time, and prothrombin time, all of which were shortened. There were also small but significant increases in the number of blood platelets and levels of total lipids and ester cholesterol of blood, liver and heart. The supplements of essential fatty acids (linoleic and linolenic), inositol, vitamin B_{12} and hydrolyzed glucose-cyclo-acetoacetate counteracted partially (but not completely) the shortening of the coagulation factors, although the effect of linoleic acid here was of questionable significance. The authors suggest that the increase in the number of blood platelets of the rabbits fed the saturated fat (which was prevented somewhat by the supplements of essential fatty acids, inositol, vitamin B_{12} or glucose-cyclo-acetoacetate) might be responsible for the increased coagulability of blood.

Although the magnitude of some of the differences reported was not great, the differences are of sufficient interest to warrant further investigation. These experiments emphasize the complexity of the relationships between dietary fat and thrombosis and indicate that many factors may be involved, of which the ones investigated may represent only a few. Moreover, these experiments indicate the difficulty of comparing results from several laboratories where these factors may vary.

Before extensive surveys of coagulation in man with respect to dietary fats are undertaken on any large scale, further animal experimentation is indicated in the light of this report by Nath and Saikia. One urgent question is the relationship between the cholesterogenic effect on serum by saturated fats and their effect on thrombosis and fibrinolysis. It is important to know whether these two effects of dietary fat are interrelated or merely coincidental. As far as this reviewer is aware a direct effect of cholesterol on coagulation or fibrinolysis has not been demonstrated. This question would appear to be of paramount importance, and the report of Nath and Saika emphasizes this need.

GLYCERIDE SYNTHESIS DURING FATTY ACID ABSORPTION

Triglyceride formation has been demonstrated during intestinal absorption of fatty acids in vitro. Homogenates of intestinal mucosa failed to activate free glycerol, indicating it is probably not a triglyceride precursor.

For over half a century it has been known that fatty acids ingested as simple esters appear in triglycerides in the thoracic duct chyle (T. P. Hilditch, *The Chemical Constitution of Natural Fats, second edition, p. 368. Wiley and Sons, New York (1947)*). Now, J. M. Johnston (*J. Biol. Chem.* **234,** *1065 (1959)*) has adapted an *in vitro* technique for the study of esterification of fatty acids during their passage through the intestinal wall. Since glycerol fed with fatty acids is not found in chyle triglycerides (R. Reiser *et al., Ibid.* **194,** *131 (1952)*), the question of the source of the glycerol for triglyceride synthesis in the intestinal mucosa has been the subject of an *in vitro* study by G. C. Buell and Reiser (*Ibid.* **234,** *217 (1959)*).

In his study of fatty acid absorption *in vitro*, Johnston (*loc. cit.*) used everted sacs of small intestine from the golden hamster, a preparation that has been used so successfully for studying amino acid and carbohydrate absorption. The mucosal side of the intestinal segment was bathed in 25 ml. of a solution containing C^{14}-labeled palmitic acid-albumin complex and glucose. The serosal side was bathed in a buffered solution containing only glucose. The experimental procedure permitted the removal of samples at intervals during the incubation (T. H. Wilson, *J. Applied Physiol.* **9,** *137 (1956)*).

The sacs were incubated for 2.5 hours at 37°C and aliquots of the mucosal and serosal solutions removed at half-hour intervals. Lipids were extracted from each aliquot of the serosal and mucosal solutions and from the intestine itself at the end of the experiment. Free and esterified fatty acids were separated and the radioactivity of each fraction was determined. Finally, the esterified fatty acids from the serosal solution were dissolved in acetone and subjected to chromatography on a silicic acid column to ensure that esters other than triglycerides were removed.

From 2.5 to 7.5 per cent of the radioactivity was transferred from the mucosal to the serosal side of the everted intestine and 11 per cent was found in the intestinal wall. Of the activity remaining on the mucosal side, 84 per cent was recovered as free fatty acids and 16 per cent as esterified fatty acids; the respective figures for the intestinal wall were 20 and 80 per cent, and for the serosal solution 13 and 87 per cent. Thus most of the fatty acids passing through the intestinal wall were esterified. The chromatographic separation of the esters indicated that these were almost exclusively di- and triglycerides with by far the greatest portion of the radioactivity being in the triglycerides.

The investigation by Buell and Reiser (*loc. cit.*) was concerned with the precursors of glyceride-glycerol in the intestinal mucosa. They used pig intestine as their starting material. The mucosa was removed and homogenized in 0.6 per cent sodium chloride. The homogenate was then centrifuged at low speed to remove cell debris and the supernatant solution was used as a source of the enzymes required for triglyceride synthesis. In some instances the preparation was centrifuged a second time to remove mitochondria as well.

The incubation mixture contained, besides the intestinal mucosa homogenate, palmitic acid, reduced diphosphopyridine nucleotide, coenzyme A, glutathione, adenosine diphosphate, magnesium chloride, phosphate buffer (pH 7.0), aldolase, and uniformly C^{14}-labeled fructose diphosphate. The reaction

mixture was incubated under anaerobic conditions for two hours at 40°C, after which the enzyme was inactivated by boiling. The lipids were then removed by solvent extraction, dried, and dissolved in ether. Phospholipids, free fatty acids and monoglycerides were removed and the remaining lipid was identified as triglyceride by a chromatographic technique. The triglyceride was then dissolved in Skellysolve B, plated, and the radioactivity determined.

From 20 to 35 per cent of the radioactivity of the labeled fructose 1,6-diphosphate was incorporated into the isolated triglycerides, indicating clearly that this compound serves as a precursor of glyceride-glycerol. In order to determine whether free glycerol or other glycerol precursors were incorporated into glycerides by the intestinal mucosa, unlabeled precursors were added to the complete incubation mixture. If an unlabeled substance were a precursor of glyceride-glycerol, less of the radioactivity from fructose diphosphate whould be incorporated into the triglycerides.

The addition of either dihydroxyacetone phosphate or L-alpha-glycerophosphate to the incubation mixture reduced the amount of radioactivity incorporated by 30 to 65 per cent. However, when free glycerol was added, no significant reduction in the radioactivity of the isolated triglycerides was observed. The authors conclude, therefore, that intestinal mucosa, in contrast to liver (E. P. Kennedy, *Ann. Rev. Biochem.* **26,** *123 (1957)*), does not contain glycerol kinase and that L-alpha-glycerophosphate is the immediate precursor of glyceride-glycerol. Thus it is not surprising that glycerol fed with free fatty acids does not appear in lymph triglycerides.

The results of these two *in vitro* studies are in accord with previous observations on intact animals and add to the evidence that triglyceride synthesis in the intestinal wall is an important step in fatty acid absorption.

THYROXINE ANALOGUES AND CHOLESTEROL METABOLISM

A number of thyroxine analogues were tested for their effects upon cholesterol metabolism. Diiodothyroacetic acid and diiodothyroformic acid seemed most effective without altering other metabolic functions.

Recently, the effect of L-thyroxine on concentrations of lipoprotein and cholesterol in the serum was reviewed (*Nutrition Reviews* **17,** *303 (1959)*). Thyroxine was capable of decreasing by a considerable degree the concentration of cholesterol and lipoproteins in the serum of a group of schizophrenic patients. However, it had undesirable effects upon the pulse rate and it decreased the diastolic blood pressure. Theoretically at least, some agent which could be administered in addition to a normal diet might prove to be effective in decreasing the serum concentration of cholesterol in persons found to have high values.

Unfortunately, most of the analogues of thyroxine which have been capable of altering lipid metabolism also have effects which would be undesirable in patients with coronary vascular disease. These include an increased metabolic rate, tachycardia, a rise in the pulse pressure and psychomotor stimulation. The exact mechanism of the thyroid hormone itself is not understood, but recent investigations have demonstrated that some analogues may manifest predominantly one action but relatively little of another.

In an effort to determine whether some of the available analogues of thyroxine would alter the metabolism of cholesterol without undue metabolic effects, W. R. Ruegamer, M. E. Alpert and F. R. Silverman (*Endocrinology* **66,** *160 (1960)*) conducted a study

in young albino rats. The experimental design was precise and the animals were housed in an air-conditioned laboratory which facilitated accurate calorimetric studies. They were given one of three diets, a commercial dog chow, a diet high in fats and cholesterol with added cholic acid, or the same high-fat diet with an increase in the amount of protein from 16 per cent to 19 per cent at the expense of carbohydrate. The thyroxine derivatives employed were as follows: 3,5-diiodothyronine; 3,5,3′-triiodothyronine; 3,5,3′,5′-tetraiodothyronine; 3,5-diiodothyroacetic acid; 3,5,3′-triiodothyroacetic acid; 3,5,3′,5′-tetraiodothyroacetic acid; 3,5,3′-triiodothyropropionic acid; and 3,5,3′,5′-tetraiodothyropropionic acid.

In the first experiment, 11 groups of rats with eight in each group were studied. One group was fed a commercial dog chow and the other ten groups were given the high-fat diet. Nine of these ten received one of the above-mentioned thyroxine analogues. These were given by intraperitoneal injection each day in a dose of 0.5 micromoles per 100 g. of body weight. At the end of the injection period the rate of oxygen consumption in the animals was determined and blood samples were obtained for cholesterol determinations. The livers were removed and samples taken for determination of cholesterol concentration.

In the second experiment, the quantity of protein in the diet was increased to 19 per cent, but otherwise it was identical. However, several additional analogues of thyroxine were employed in comparison with thyroxine itself. These included 3,5,3′-triiodothyroethylamine; 3,5-diiodothyroformic acid; 3,5,3′-triiodothyroformic acid; 3,5,3′,5′-tetraiodothyroformic acid; and 3,5-diiodothyropropionic acid.

In the first experiment it was apparent that each of the analogues had a significant effect upon the hypercholesterolemia, and two of them, 3,5-diiodothyroacetic acid and 3,5,3′-triiodothyroacetic acid, reduced the hepatic concentration of cholesterol significantly. However, 3,5,3′-triiodothyroacetic acid caused a significant inhibition of weight gain and a significant increase in consumption of oxygen.

In the second experiment, it was evident that the increased allowance of protein resulted in a lesser degree of hypercholesterolemia. It also increased the rate of growth of the control animals. Of the various analogues used in this experiment, significant decreases of the plasma cholesterol occurred with thyroxine or with 3,5-diiodothyroformic acid. The latter also had a significant effect in decreasing the concentration of cholesterol in the liver. It did not alter the rate of weight gain or the consumption of oxygen significantly.

The authors concluded that, of the analogues tested, 3,5-diiodothyroacetic acid and 3,5-diiodothyroformic acid showed the greatest preferential effect upon lipid metabolism without significantly influencing oxygen consumption or growth of the animals.

Much remains to be learned concerning the metabolism of cholesterol and other lipids. For example, an interrelationship has been demonstrated between the thyroid hormones and androgens. L. Hellman *et al.* (*J. Clin. Endocrinol. Metab.* **19,** *936* (*1959*)) demonstrated that myxedematous patients excreted subnormal quantities of androsterone. Administration of triiodothyronine to these patients resulted in an increase of androsterone and had a similar effect in normal subjects. Further demonstration of the relationship between those two hormonal systems was obtained by administering androsterone to myxedematous patients, which resulted in a decrease in serum cholesterol and, in one patient, an increase in rate of oxygen utilization.

The study of thyroxine derivatives demonstrated clearly that some of the effects of thyroid hormone could be separated quanti-

tatively from others. However, it remains to be determined whether this separation will be apparent in humans, and if so, whether these substances will be harmless. It would be of great interest to learn whether the hypocholesterolemic effect of thyroxine analogues is accompanied by an alteration in the formation and excretion of androgens.

COMPARATIVE NUTRITIVE VALUE OF COMMON EDIBLE FATS

There appears to be little difference in the nutritive value of most common edible fats even in prematurely weaned animals.

A great many studies have been conducted on the relative nutritive properties of various animal fats and vegetable oils, especially in comparison to those of butterfat (*Nutrition Reviews* **1,** *122, 358 (1943)*; **2,** *165, 267, 351 (1944)*; **3,** *271 (1945)*; **4,** *140 (1946)*; **8,** *232 (1950)*; **14,** *305, 349 (1956)*; **16,** *331 (1958)*). Although the general conclusion reached in most of these studies has been that there is little difference in the nutritive value of the common fats (when compensated for digestibility), there has not been agreement on certain experimental conditions.

For example, it has been claimed (E. J. Schantz, R. K. Boutwell, C. A. Elvehjem and E. B. Hart, *J. Dairy Sci.* **23,** *1205 (1940)*) and denied (H. J. Deuel, Jr., and E. Movitt, *J. Nutrition* **29,** *237 (1945)*) that even if no difference exists in animals studied from the normal weaning age, butterfat is superior in those weaned prematurely. Moreover, there are indications that the other dietary constituents may influence the relative nutritive values of the fat (Boutwell, R. P. Geyer, Elvehjem and Hart, *Ibid.* **26,** *601 (1943)*).

In an attempt to resolve some of these differences, E. W. Crampton, R. K. Shaw, V. G. Mackay and D. C. Schad (*J. Nutrition* **70,** *81 (1960)*) have measured the nutritive value of several common fats for prematurely weaned animals. One-hundred and eight puppies, 172 pigs and 266 guinea pigs were weaned at ten, 14 and three days, respectively, and divided into groups which were placed on low-fat diets largely composed of cereals and skim milk powder with adequate vitamin and mineral supplements and 20 per cent of 15 different vegetable or animal fats. Digestibility was determined by the chromic oxide method and records of weight and feed intake were maintained for four weeks for the other animals.

Weight gains were similar for most groups of animals of each species, regardless of the type of dietary fat. However, some growth depression was seen for pigs on the fish, rapeseed and coconut oil diets, and for guinea pigs on the hydrogenated fish oil. When the mean weight gains of the five groups of each species fed butterfat diets were compared with those of all groups on other types of fat, it was found that there was 18, 10 and 14 per cent greater gain on butterfat than on other types.

These differences appeared to be related to a greater feed intake by the butterfat groups, a finding which had been ascribed by Deuel and Movitt (*J. Nutrition* **27,** *399 (1944)*) to flavor preference. It is also possible, however, that these differences were related to the rapid intestinal absorption of butterfat, as reported by H. J. Thomasson (*J. Nutrition* **59,** *343 (1956)*). Similarly, the lower growth rates of groups on fish, rapeseed and coconut oils could be equated to lower digestibility of these fats,

in agreement with Thomasson (*loc. cit.*). Thus, when these factors were taken into consideration, no significant differences in nutritional value of butterfat as compared to other animal and vegetable fats could be observed. This conclusion was also confirmed by a comparison of feed efficiency (average gain per 1000 digested calories).

No answer was obtained in these experiments to the question of the effect of dietary carbohydrate on nutritional value of the fats, since the carbohydrate source of all groups was of mixed origin. However, it seems clear that age of weaning has little or no effect on the comparative nutritional value of the different fats.

PLASMA AMINO ACIDS AND DIETARY PROTEIN

A method, based on measurement of changes in plasma concentrations of amino acids following a test meal, is proposed for predicting the sequence in which the amino acids of a protein become limiting for tissue synthesis.

The nutritive value of a dietary protein depends upon how closely the relative quantites of amino acids reaching the tissues of a subject ingesting it correspond with the relative quantities of amino acids required by the tissues. A nutritionally perfect protein would be completely digestible, would have a ratio of dispensable to indispensable amino acids identical with that required by the subject (a ratio as yet undetermined), and a pattern of indispensable amino acids that coincided with the pattern of amino acid requirements of the subject.

The last of these criteria is undoubtedly most important because a measure of the extent to which the pattern of indispensable amino acids in a protein deviates from a standard approaching the ideal gives a fairly good estimate of the nutritive value of that protein. This is the basis for the chemical score method of estimating protein quality devised by R. J. Block and H. H. Mitchell (*Nutrition Abstracts & Reviews* **16,** *249 (1946)*). They used the amino acid pattern of the proteins of whole egg as a reference standard.

Recently, two attempts have been made to design amino acid patterns that would serve specifically as reference standards for human nutrition studies (*Protein Requirements. Food and Agriculture Organization Nutritional Studies No. 16 (1957)*; *Evaluation of Protein Nutrition. National Academy of Sciences-National Research Council Publication No. 711 (1959)*). These developments have made it possible to estimate reasonably well the nutritive value of a protein from a knowledge of its amino acid composition. They have not, however, obviated the need for a rapid, simple and accurate method of determining the nutritive value of a protein experimentally.

Among the experimental methods that have been used to estimate the nutritive value of a protein (*Nutrition Reviews* **10,** *33 (1952)*) are the nitrogen balance technique for the classical determination of biological value or net protein utilization (Mitchell, *J. Biol. Chem.* **58,** *873, 905 (1923)*), the rat growth assay for the determination of protein efficiency ratio (T. B. Osborne and L. B. Mendel, *Ibid.* **32,** *369 (1917)*), and carcass protein or moisture analyses (D. S. Miller and A. E. Bender, *Brit. J. Nutrition* **9,** *382 (1955)*). The nitrogen balance technique is time-consuming and inconvenient for human studies. The carcass analysis method is applicable only to animal studies, while the protein efficiency ratio method is applicable only to growing subjects and its value is extremely limited unless the many variables that influence it are rigidly controlled (Bender, *Proc. Nutrition Soc.* **17,** *85 (1958)*),

a near impossibility in studies on human subjects. Therefore, new techniques that show promise as a means of estimating directly the nutritive value of proteins for human subjects are bound to interest nutritionists (*Nutrition Reviews* **11,** *347 (1953)*).

An investigation by J. B. Longenecker and N. L. Hause on the relationship between plasma amino acid concentrations and the composition of the ingested protein (*Arch. Biochem. Biophys.* **84,** *46 (1959)*) should elicit such interest. The basic assumptions underlying the investigation are that the amino acid composition of the blood plasma of animals fed a single test meal should depend not only upon the amino acid composition of the dietary protein but also upon the rate and extent to which it is digested and upon the rate and extent to which the various amino acids are metabolized by the body. Thus plasma amino acid concentrations following a test meal should reflect the nutritional adequacy of the ingested protein.

It has long been hoped by nutritionists that plasma amino acid concentrations might be useful in assessing the nutritional status of the individual and the nutritive value of proteins. Several papers are cited by Longenecker and Hause (*loc. cit.*) indicating that plasma amino acid concentrations after the ingestion of a protein are roughly proportional to the amounts of amino acids supplied by the protein. Also, in a study of the protein-sparing effect of carbohydrate, H. N. Munro and W. S. T. Thompson (*Metabolism* **2,** *354 (1953)*) demonstrated that, upon administering glucose to human subjects in the fasting state, the plasma amino acid concentrations decreased approximately in proportion to the accepted amino acid requirements, suggesting that they were used for protein synthesis in these proportions.

Also, after reviewing the literature on blood plasma amino acid concentrations, H. J. Almquist (*Arch. Biochem. Biophys.* **52,** *197 (1954)*; *The Amino Acid Handbook, p. 152. C. C. Thomas (1956)*) concluded that the amino acid pattern obtained after the ingestion of a protein frequently deviated from that expected. He noted, in particular, that the concentration of an amino acid which was deficient in the ingested protein was usually inordinately low in the plasma. He suggested that these deviations could be explained if the amino acids were used in proportion to the requirements and that the amino acid pattern of the plasma would resemble that of the protein ingested only if the protein were of high nutritive value. From these observations he concluded that the plasma amino acid pattern might provide a sensitive index of protein quality.

However, until the publication of the paper by Longenecker and Hause, no method had been devised for relating plasma amino acid concentrations to the composition of the ingested protein in a way that might prove useful for estimating the nutritive value of a protein.

The experiments from which their method was developed were performed on adult mongrel dogs weighing about 10 kg. Food was withheld from them for 18 hours; then they were fed 200 g. of a test diet containing 32 per cent protein (wheat gluten, gelatin or casein), 10 per cent cottonseed oil and 58 per cent sucrose. Immediately before the meal and at hourly intervals for five hours afterward, 25 ml. of blood was removed from each animal. The erythrocytes were centrifuged off and the amino acid composition of a protein-free filtrate of the plasma was determined by ion exchange chromatography, except for tryptophan which was determined by microbiological assay. Nine trials were run, three with wheat gluten, two each with gelatin and casein, one with wheat gluten supplemented with lysine, and one with gelatin supplemented with tryptophan.

The concentrations of the essential amino acids in the plasma of each dog at each hourly interval were tabulated, but no consistent relationship between the amino acid

composition of the protein ingested and that of the blood plasma was evident. In an effort to bring some order out of what appeared to be chaos, the authors calculated what they called "plasma amino acid ratios." This was done by averaging the five hourly plasma values for each amino acid and subtracting the corresponding fasting value to give a measure of the change in the plasma concentration of each amino acid following the ingestion of the test meal. Each of the values so obtained was divided by the corresponding value for the requirement of the dog, and this multiplied by 100 was taken as the plasma amino acid ratio for that amino acid.

For example, after the ingestion of wheat gluten, the average plasma concentration for leucine was 3.07 mg. per 100 ml. Subtracting the fasting value of 1.05 gave a value of 2.02 for the change in concentration. This divided by 8.5 (the requirement of the dog for leucine being 8.5 g. per 16 g. of nitrogen) and multiplied by 100 gave a value of 23.8. The values obtained for different amino acids in the various experiments ranged from −65 to 58.

It was assumed that the most limiting amino acid would yield the smallest positive or the largest negative number and that a tabulation of the plasma amino acid ratios in ascending order would reveal the sequence in which the amino acids of the test protein became limiting for the dog. The sequences obtained for the more limiting amino acids of gelatin and wheat gluten corresponded fairly well with the sequences calculated by the chemical score method. The sequences for the less limiting amino acids of these two proteins and for casein did not show such close correspondence.

The lack of correspondence should not be taken as a severe criticism of this procedure because there is no evidence that the sequences obtained by the chemical score method provide a reliable standard for comparison. In fact, the greatest obstacle to the development of this method is the lack of information which will permit an estimate of its reliability. The final proof of the reliability of such a method is the accuracy with which it can be used to predict the order in which amino acids must be added to a diet containing a low level of the protein in order to stimulate growth or increase nitrogen retention.

If the method provides an accurate estimate of the sequence in which the amino acids in a protein become limiting, it should be possible to show, by using protein-depleted dogs fed a diet containing a low level of protein, that nitrogen retention increases stepwise as the essential amino acids are added one after another in the sequence predicted from the estimated plasma amino acid ratios. For the growing animal, it should be possible to demonstrate stepwise increases in growth rate using the same procedure.

Many questions can be raised about factors that might influence the reproducibility of the plasma amino acid ratios determined by the method of Longenecker and Hause; for example, the effect of the type of diet on the rate of stomach emptying (C. Peraino *et al., Canad. J. Biochem. Physiol.* **37,** *1475 (1959)*), the effect of changes in the rate of blood flow, the influence of rapid protein synthesis in the growing animal, and the effect of inaccuracies in the estimated amino acid requirements of the subjects. The averaging of five hourly samples undoubtedly helps to remove much of the variability, and further investigation of this technique will answer many of these questions.

In the meantime, the method should be studied carefully because it offers promise as a procedure for determining directly the sequence in which the amino acids of a protein become limiting for growth and maintenance, and thus offers promise as a direct method for estimating the nutritive value of proteins for human subjects. With further development, it may prove of value as a technique for determining individual amino acid requirements.

NOTES

Possible Growth Inhibitor in Uncooked Peas

E. W. Kienholz, L. S. Jensen and J. McGinnis have reported "Improvement in nutritional value of peas by cooking" under this title (*Proc. Soc. Exp. Biol. Med.* **102,** *35 (1959)*). They point out that field peas are deficient in methionine, but that chicks fed diets containing abundant peas do not grow maximally even with added methionine. Their studies were designed to discover the cause of the deficient growth.

Alaska peas were ground, mixed with water, autoclaved and then oven-dried at 60°C and reground. Autoclaved peas were similarly prepared but without water. Uncooked frozen peas (Thomas Laxton variety) were oven-dried at 60°C.

The authors fed diets containing high levels of peas, with added methionine, vitamins and minerals, to day-old chicks and turkey poults for two weeks. The growth rate of the turkeys was greatly increased by cooking the peas, especially with water, and was comparable to that on the control corn-soy diet. Increasing the caloric content of the diets by adding tallow had less effect than cooked peas in a diet of fewer calories.

Kienholz, Jensen and McGinnis conclude that heat inactivates a growth inhibitor in peas. They indicate that it is not a trypsin inhibitor nor is it beta-aminopropionitrile. However, they report only preliminary attempts to concentrate the material.

It is unfortunate that no record was made of dietary intake, which was ad libitum; thus the growth value of different diets is difficult or impossible to assess. Also, no pathologic studies were performed. Interference with growth is a gross result compared with histologic changes, which often provide a clue to the nutritional mechanisms involved.

One looks to future work to compare the growth value of isocalorically consumed diets, to define the lesions if any, and to fractionate and identify the growth inhibitor if such it be.

Nutritional Excess in Infancy and Childhood

Parents of today are exhorted on television and in the press to feed their children vitamins, minerals, meat, milk, cereals and many other items. W. A. Cochrane (*Canad. Med. Assn. J.* **81,** *454 (1959)*) points out that physicians have done little to restore reason, especially with respect to infants. It is thought generally that a growing baby is a healthy baby, equating size with health. However, according to M. L. Johnson, B. S. Burke and J. Mayer (*Am. J. Clin. Nutrition* **4,** *231 (1956)*), 10 per cent of all American children are overweight. H. V. Merideth (*Am. J. Dis. Child.* **62,** *909 (1941)*) found that infants of one year were 7 per cent taller than those of the nineteenth century, and boys of nine to 14 years were 6 to 8 per cent taller and 12 to 15 per cent heavier.

Maximal rate of growth may not be equivalent to optimum health and greatest longevity, if information from animals proves to be applicable to man. For example, increasing dietary protein of young rats results in an earlier appearance of kidney lesions (G. C. Kennedy, *Brit. Med. Bull.* **13,** *67 (1957)*).

A possible detrimental effect of excess is seen in Great Britain and Switzerland where a condition has appeared in infants known as "idiopathic hypercalcemic syndrome". These children grow rapidly but develop constipation, vomiting, abnormal renal function, and hypertension. They have been fed foods containing large quantities of vitamin D, and have elevated concentrations of calcium in their serum. British cod liver oil contains twice as much vitamin D as ours, and their fortified milk three and one-half times as much (R. Lightwood *et al.*, *Brit. Med. J.* **2,** *149 (1956)*).

These considerations seem to indicate that any changes which we might propose in our dietary routine must be evaluated fully before they are advocated for general adoption.

Bisalbuminemia

The presence of abnormal proteins in the blood is being reported more and more frequently as methodology becomes more sensitive and definitive. G. Franglen *et al.* (*Lancet* **I**, *307* (*1960*)) have recently reported the presence of a second albumin in the serum obtained from a 16-year-old boy who had an unusual dermatological condition of his hands. This albumin, called a bisalbumin, migrated on paper under an electric potential a bit slower than normal albumin. However, it moved faster than the gamma-globulin. The boy's hands were cold and blue even in summer and had a horny layer over the knuckles. Also he had an inability to straighten the fingers involved. No acrosclerosis or any form of Raynaud's disease was present. The authors concluded that this was an hereditary albumin abnormality, since such conditions were also noted in the hands of the boy's father and his paternal grandfather was reported to have cold blue hands. The abnormal protein was also found in the blood of four of the patient's relatives.

The investigators believed the abnormal protein to be an albumin since zone electrophoresis on paper, cellulose acetate, and agar revealed well-defined double bands which migrated similarly to albumin and faster than the globulins. Also the alpha-globulin could be identified as clearly separated from the abnormal albumin. Secondly, using moving boundary analysis, a well-defined hump could be seen on the posterior slope of the peak and the alpha-globulin area was clearly visible behind it. Thirdly, dyes believed to couple specifically with albumin were found to be bound to both of the bands noted. Finally, using electrophoresis on starch blocks, the two bands were shown not to differ immunologically from normal human albumin.

The abnormal albumin represented about 50 per cent of the total albumin and this proportion was remarkably constant. In fact, either the members of the family had a level of approximately 50 per cent of the total serum albumin or the abnormality did not appear at all in the blood. C. G. Bergstrand and B. Czar (*Scand. J. Clin. Lab. Invest.* **9**, *277* (*1957*)) have reported the presence of a protein which migrates between the albumin and alpha-globulin in the serum of fetuses obtained from legal abortions up to the nineteenth week. This component amounted to about 10 per cent of the normal albumin level. However, the present investigators suggest only tentatively that the abnormal albumin they detected may represent a persistence of fetal type albumin, even though it was considerably higher in concentration than that reported by Bergstrand and Czar.

While these authors were tempted to correlate the presence of the unusual skin condition with the protein abnormality, they noted that four other members of the family, two males and two females, had the abnormal albumin but did not show any dermatalogical defect.

With better techniques of fractionating proteins becoming available, various hereditary abnormalities not previously observed should be detected. Whether or not these produce changes in the body's economy still has to be decided.

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